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The influence of mechanical compression on the induction of osteoarthritis-related biomarkers in articular cartilage explants

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Summary

Objective: Macromolecules of the articular cartilage extracellular matrix released into synovial fluid, blood, or urine can serve as potentially useful biomarkers of the severity of osteoarthritis (OA). Biomechanical factors play an important role in OA pathogenesis, yet their influence on biomarker production is not well understood. The goal of this study was to examine the hypothesis that dynamic mechanical stress influences the release of these biomarkers from articular cartilage.

Methods: Explants of porcine cartilage were subjected to dynamic compression at 0.5 Hz for 24 h at stresses ranging from 0.006 to 0.1 MPa. The concentrations of cartilage oligomeric matrix protein (COMP), keratan sulfate (KS measured as the 5D4 epitope), total sulfated glycosaminoglycan (S-GAG), and the KS (keratanase-digestible) and chondroitin sulfate (CS) (chondroitinase-digestible) fractions of S-GAG were measured. Radiolabel incorporation was used to determine the rates of proteoglycan and protein synthesis.

Results: The magnitudes of mechanical stress applied in this study induced nominal tissue strains of 4–23%, consistent with a range of physiological to hyperphysiologic strains measured *in situ*. COMP release increased in proportion to the magnitude of dynamic mechanical stress, while KS, CS and total S-GAG release increased in a bimodal pattern with increasing stress. Protein and proteoglycan synthesis were significantly decreased at the highest level of stress.

Conclusion: Mechanical stress differentially regulates the turnover of distinct pools of cartilage macromolecules. These findings indicate that mechanical factors, independent of exogenous cytokines or other stimulatory factors, can influence the production and release of OA-related biomarkers from articular cartilage.

Key words: Proteoglycan, Cartilage oligomeric matrix protein, COMP, Keratan sulfate, Chondroitin sulfate, Biomechanics, Mechanical compression, Arthritis.

Introduction

Osteoarthritis (OA) is a painful and debilitating disease of the synovial joints characterized by degenerative changes in the structure, composition, and mechanical function of the articular cartilage, subchondral bone, and other joint tissues[1]. A hallmark of the disease is the progressive degradation of the articular cartilage extracellular matrix that occurs, despite the increased biosynthetic activity of the chondrocytes, suggesting that the normal balance of anabolism and catabolism of the extracellular matrix has been disrupted[2–4]. The extracellular matrix consists primarily of type II collagen[5] and the large aggregating proteoglycan, aggrecan[6]. Aggrecan consists of a protein core to which are attached the sulfated glycosaminoglycan (S-GAG) chains, keratan sulfate (KS) and chondroitin sulfate (CS)[7]. Smaller amounts of other collagen species (e.g., types VI, IX, and XI), smaller proteoglycans, and other macromolecules such as cartilage oligomeric matrix protein (COMP), hyaluronan, fibronectin, and tenascin are also present in the extracellular matrix[8].

These extracellular matrix molecules or their fragments can be useful as biological markers, or "biomarkers", to assess the severity of joint disease by minimally invasive means[9–12]. Biomarkers of arthritis are defined as macromolecules originating from joint structures whose levels in synovial fluid, blood, or urine reflect locally occurring metabolic events in the joint[12]. Several of these molecules have been investigated as potential biomarkers of matrix metabolism in OA. Monoclonal antibodies (mAbs) to cartilage macromolecules and various carbohydrate epitopes in the glycosaminoglycans found in cartilage have been valuable in studying cartilage metabolism, as well as alterations in proteoglycan structure and function under conditions of repair or degeneration[13]. For example, COMP is a glycoprotein composed of five identical subunits[14] that has been identified as a potential marker of the severity and progression of OA. The concentration of COMP in the serum or synovial fluid increases in clinical and animal models of OA in relation to the severity of disease as well as the number of joints affected[10,15–21]. The mAb 5D4 recognizes KS, and reacts to an epitope of seven repeating units of disulfated disaccharides[22]. Increases in the synovial fluid and serum levels of 5D4 are associated with OA[10,23–26]. A more general

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measure of proteoglycan, total S-GAG, also increases in synovial fluid in clinical[7,27] and animal models[28] of OA.

Despite the extensive consequences of OA, the etiopathogenesis of this disease is not fully understood and appears to involve a complex interaction of biomechanical stress as a cofactor together with the local biochemical environment[1,29–31]. Both clinical and animal studies have provided strong evidence that mechanical factors can initiate and contribute to the imbalance of cartilage metabolism in OA. Alterations in the normal pattern of joint loading may predispose to OA and may be caused by a variety of factors such as immobilization, joint instability, overuse, trauma, injury, or obesity[32–34]. Studies of OA progression following meniscal or ligamentous instability link alterations in joint loading and kinematics to specific biomechanical changes at the tissue level that may predispose the joint to OA, such as decreased cartilage stiffness (tensile and compressive moduli) and increased hydraulic permeability[35–37].

To better understand the role of mechanical stress on articular cartilage, several *in vitro* models have been developed that allow the application of mechanical stress to articular cartilage in a controlled biochemical environment. In such models, continuous or intermittent compression can alter the biosynthetic rates of proteoglycan and collagen[35,38–47]. The consensus of these studies is that static compression suppresses matrix biosynthesis, while cyclic and intermittent loading can either stimulate or suppress matrix synthesis, depending on the frequency or magnitude of loading. High rates or magnitudes of stress can induce an "injurious" response that has been associated with increased degradation, cell death, and the production of matrix metalloproteinases[40,48–52]. These responses have been reported over a wide range of loading magnitudes and frequencies, and exhibit a stress–dose dependency[53].

Evidence exists for an interaction between mechanical stress and OA-related biomarker release. *In vivo* studies show that physical exercise (running on a treadmill for 60 min or playing soccer for 90 min) can increase the levels of the 5D4 epitope[54], as can running in horses[55]. However, other studies have reported no statistically significant difference in serum KS levels in marathon runners (before and after a 42-km marathon)[56,57] or in patients with arthritis in response to a more chronic (3 months) conditioning activity[58]. A recent study has shown that serum COMP levels are increased immediately following 30 min of walking in healthy subjects[59]. Several *in vitro* studies also provide evidence for a link between dynamic loading of cartilage and the release of various biomarkers of OA. Dynamic compression of cartilage in culture increased immunolabeling for the 3B3(−) epitope of CS, which is believed to be an early indicator of alterations in cartilage metabolism[60] and a marker of cartilage development[41]. Other studies have shown increased 3B3(−) labeling in adult bovine cartilage under a relatively severe compression regimen (5 MPa, 24 h at 0.5 Hz) that was also associated with cell death and tissue damage[40]. Cyclic mechanical compression or tension can upregulate the expression of the COMP gene at different frequencies and magnitudes of stress *in vitro*[61–63]. These few studies suggest an interaction between mechanical stress and OA-related biomarkers; however, the dose–response relationship between the magnitude of dynamic compression and the synthesis and release of these biomarkers is not fully understood.

The goal of this study was to test the hypothesis that cyclic mechanical compression of cartilage alters the release of biomarkers of OA in a manner that depends on the applied stress magnitude. A range of compressive loads was applied to articular cartilage explants to induce physiologic and hyperphysiologic magnitudes of tissue strain (deformation). The release of COMP, the proteoglycan epitope 5D4 (KS), and total S-GAG, as well as the KS (keratanase-digestible) and CS (chondroitinase-digestible) fractions of S-GAG, into the media was measured. Radiolabel incorporation of ^{35}S-sulfate and ^{3}H-proline was used to determine the rates of proteoglycan and protein synthesis, respectively, as a function of stress magnitude.

Materials and methods

ARTICULAR CARTILAGE EXPLANTS

Full-thickness explants of articular cartilage were harvested from the femoral condyles of skeletally mature female pigs (1.5–3 years old) using a 5 mm dermal biopsy punch (Miltex Inc, Bethpage, NY) and separated from the underlying bone. The explants were harvested in adjacent pairs to allow site-matching of control (uncompressed) and compressed specimens to reduce site-to-site variability among explants. Before testing, the explants were allowed to stabilize in culture for 72 h at 37 °C, 5% CO_2, in a 48-well plate containing 1 ml/well of standard culture medium consisting of Dulbecco's Modified Eagle Medium (Gibco, Gaithersburg, MD) with 10% fetal bovine serum (heat inactivated at 56 °C for 30 min) (Sigma Chemicals, St. Louis, MO), 0.1 mM modified eagle medium non-essential amino acids (Gibco), 100 U/ml penicillin/streptomycin (Gibco), 10 mM N-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid buffer (Gibco), and 37.5 μg/ml ascorbate-2-phosphate[64]. Synthetic rates and levels of biomarkers released into the culture media were normalized to the wet weight of cartilage measured before compression.

MECHANICAL COMPRESSION EXPERIMENTS

To determine the effects of mechanical compression on biomarker release, individual explants were dynamically loaded in unconfined compression at stresses of 0.006–0.1 MPa using a modified version of the Biopress™ system, a computer-controlled instrument for compressing tissue explants (Flexcell International, Hillsborough, NC)[64–66]. Briefly, individual cartilage explants were placed in Biopress™ culture plates (Flexcell International) consisting of a Delrin™ chamber attached to the bottom of a flexible silicone rubber membrane. A range of calibrated air pressures was applied to the membrane, and the corresponding compressive stress (σ) applied to the explant was determined from the applied force (F) and the initial cross-sectional area (A) of the explant, where $\sigma = F/A$. The loads were applied at a frequency of 0.5 Hz for 24 h at 37 °C, 5% CO_2. Unloaded (control) explants were incubated for 24 h under the same culture conditions.

MEASUREMENT OF TISSUE STRAIN UNDER DYNAMIC LOADING

To determine the deformation of the explants under the range of applied stresses, a set of explants ($n = 5$) was loaded under the same conditions in an electromechanical materials testing system (EnduraTec ELF 3200, Minnetonka, MN) using a closed-loop, load-controlled test regimen. Each explant was first placed under a tare load of 10 gf and allowed to equilibrate for 1 h. Cyclic compression was applied at 0.006, 0.0125, 0.025, 0.05, or 0.1 MPa at 0.5 Hz

and allowed to reach a steady-state response for 3 h. At this point, the root-mean-square (RMS) and the range of strain amplitudes over a loading cycle were determined at steady state as a function of the applied stress.

MEASUREMENT OF BIOMARKER RELEASE

After 24 h of compression, levels of COMP and KS in the media surrounding the explants were measured by enzyme-linked immunosorbent assay. KS was measured with mAb 5D4 as previously described[10]. COMP was measured with mAb 12C4[21], which cross-reacts to porcine COMP, as previously described[67].

MEASUREMENT OF TOTAL S-GAG AND KERATANASE- AND CHONDROITINASE-DIGESTIBLE FRACTIONS OF S-GAGS

Total S-GAG was measured with the 1,9-dimethylmethylene blue (DMB) assay[68,69]. For this assay, eight standards were used, prepared with control media and CS C (Sigma Chemicals) at concentrations 0–100 μg/ml. Twenty microliters per well of standards and sample media were pipetted into a 96-well plate, and 125 μl/well of DMB (pH 3.00) was added. Optical absorbance was read at 540 nm within 5 min of DMB addition.

To determine the relative proportions of KS and CS, the predominant S-GAGs in cartilage, the media were digested using purified keratanase I (KI, *Pseudomonas* sp) and II (KII, *Bacillus* sp) or chondroitinase ABC (ABC, *P. vulgaris*) (Seikagaku, Tokyo, Japan and MP Biomedicals, Costa Mesa, CA), respectively. KI hydrolyzes monosulfated disaccharide repeats in KS, while KII hydrolyzes mono- and disulfated disaccharide repeats. ABC hydrolyzes CSs A, B, and C. Briefly, 25 μl aliquots of experimental media were incubated with no enzyme (undigested), 37.5 mU of KI, 6.5 mU of KII, 37.5 mU of KI and 6.5 mU of KII, or 37.5 mU of ABC. All digestions were performed in 0.1 M Tris acetate and 0.05 M sodium acetate digestion buffer (pH 6.5) for 4 h at 37°C. Total S-GAG concentration was measured in the undigested and digested samples using the DMB assay as described above. The difference in total S-GAG levels between the undigested and digested samples was attributed to the amount of KS or CS originally present in the samples.

MEASUREMENT OF PROTEOGLYCAN AND PROTEIN SYNTHESIS RATES

The effects of mechanical stress on matrix biosynthesis were determined using the incorporation of radioactive ^{35}S-sulfate (10 μCi/ml) and ^{3}H-proline (20 μCi/ml) as a measure of proteoglycan and total protein synthesis, respectively[35,38,44]. Radiolabel incorporation was measured for the entire culture period. At the end of each experiment, the explants were washed four times for 15 min each in phosphate buffered saline with 0.8 mM sodium sulfate and 1 mM L-proline to remove unincorporated radioactive label[44]. Specimens were incubated in 125 μg/ml papain (Sigma) at 60°C until fully digested. Four milliliters of Bio-Safe II scintillation fluid (Research Products International, Mount Prospect, IL) was added to each vial and disintegrations per minute were counted using a liquid scintillation counter (Model 1900TR, Packard Instrument Co., Meriden, CT).

For statistical analysis of biomarker concentrations in the media or radiolabel incorporation into explants, paired Student's *t* tests were performed for each stress level individually, with significance reported at the 95% confidence level ($P < 0.05$). Correlation analysis was performed using the Pearson Product–Moment Correlation test.

Results

TISSUE STRAIN UNDER DYNAMIC LOADING

Explants showed increasing peak-to-peak strain magnitudes with increasing mechanical stress magnitudes from 0.006 to 0.1 MPa, resulting in nominal tissue strains of 4–23% (Fig. 1).

RELEASE OF COMP AND 5D4 EPITOPE WITH LOAD

COMP release into the media increased with increasing magnitude of mechanical loading, with significant increases at 0.05 MPa ($P < 0.05$) and at 0.1 MPa ($P < 0.01$) compared to uncompressed explants (Fig. 2). KS release (5D4 epitope) significantly increased at 0.05 MPa, with a peak release observed at 0.0125 MPa (Fig. 3).

RELEASE OF TOTAL S-GAG AND KERATANASE- AND CHONDROITINASE-DIGESTIBLE FRACTIONS OF S-GAG WITH LOAD

Mechanical compression significantly increased total S-GAG release into the media at all magnitudes of compression above 0.006 MPa ($P < 0.01$), with the highest release at 0.0125 MPa [Fig. 4(a)].

The proportion of total S-GAG released due to total KS (KI + KII-digestible fraction) and CS (ABC-digestible fraction) paralleled the increases in total S-GAG (undigested) release into the media [Fig. 4(a and b)] as compared to site-matched, uncompressed controls. Significant increases in total KS (KI + KII digestion) were observed at 0.025–0.1 MPa ($P < 0.05$), and in CS at 0.0125–0.1 MPa ($P < 0.01$), except at 0.025 MPa [Fig. 4(b)]. The chondroitinase-digestible fraction of S-GAG contributed to the majority of the S-GAG levels in both control (68–89%) and loaded groups (72–83%). The KI-digestible fraction of

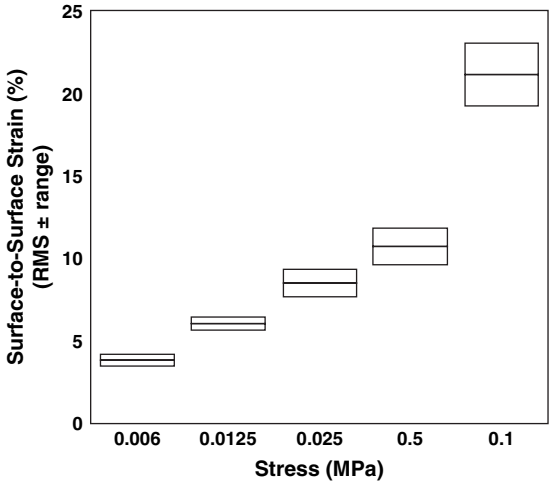

Fig. 1. Steady-state magnitudes of the RMS of strain and the range of strain during a loading cycle in explants of articular cartilage at each stress magnitude. The loads applied in this study resulted in a range of physiologic to hyperphysiologic tissue strains. Data are presented as mean ± range during 1 cycle of loading, $n = 5$.

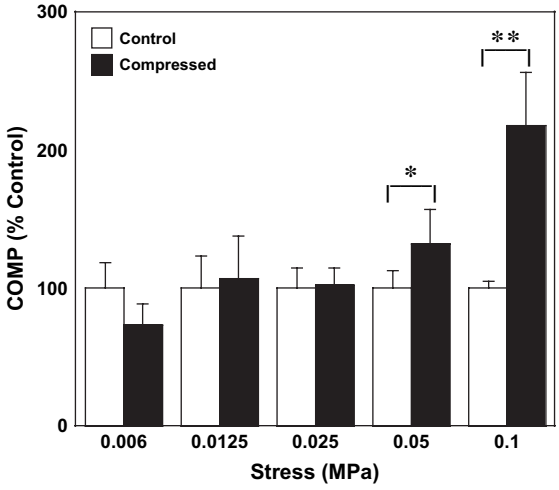

Fig. 2. COMP release into the culture media following mechanical loading of 0.006–0.1 MPa at 0.5 Hz. Results are normalized to the wet weight of the articular cartilage explants and expressed as the percent change of compressed over control values. Data are presented as mean ± S.E.M., $n = 18$, $^{*}P < 0.05$, $^{**}P < 0.01$.

S-GAG release increased significantly at 0.05 MPa ($P < 0.005$) [Fig. 4(c)], while the KII-digestible fraction increased significantly at 0.025–0.1 MPa ($P < 0.05$) [Fig. 4(d)].

PROTEOGLYCAN AND PROTEIN SYNTHESIS

No significant changes were observed in the rates of proteoglycan and protein synthesis at low magnitudes of mechanical stress (0.006–0.0125 MPa) [Fig. 5(a and b)]. Higher levels of magnitude of mechanical stress (0.025–0.01 MPa) decreased proteoglycan and protein synthesis relative to control, non-compressed samples ($P < 0.05$ for 0.1 MPa).

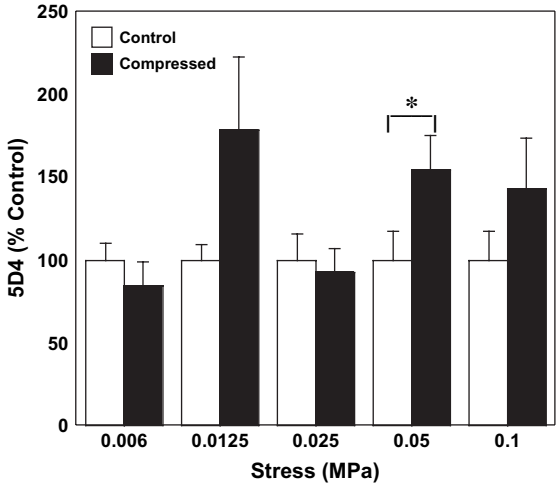

Fig. 3. KS (5D4 epitope) release into the culture media following mechanical loading of 0.006–0.1 MPa at 0.5 Hz. Results are normalized to the wet weight of the articular cartilage explants and expressed as the percent change of compressed over control values. Data are presented as mean ± S.E.M., $n = 18$, $^{*}P < 0.01$.

Discussion

The results of this study show that dynamic mechanical compression can alter the release of the OA-related biomarkers COMP, KS, and S-GAG, and alter the rates of extracellular matrix synthesis in a manner that varied with the magnitude of applied stress. These findings also indicate that the magnitude of mechanical stress can differentially regulate the turnover of distinct pools of cartilage macromolecules. These data suggest that the production and release of cartilage biomarkers *in vivo* may be influenced by the local mechanical environment of the joint, and support the hypothesis that biomechanical factors may contribute to metabolic changes in cartilage that are characteristic of OA[10,30,31,37,59].

The magnitudes of mechanical stress applied in this study were selected to induce nominal tissue strains of 4–23%, which represent a range of physiologic to hyperphysiologic strains observed in human cartilage *in vitro* or *in vivo*[70,71]. For example, previous radiographic studies have reported strains of 2–20% in femoral head cartilage under a static load of five times body weight[70], while other magnetic resonance imaging studies on healthy individuals showed mean changes in cartilage thickness of 2.8% and 4.9% after several minutes of recovery following dynamic loading (knee bends) or static loading (squatting), respectively[71]. While it is difficult to correlate directly the stress–strain parameters between our explant loading system and the *in vivo* state, we presume that the tissue strains induced *in vitro* by the applied stresses in our model represent a physiologic range from 0.006 to 0.05 MPa, while the higher stress magnitude (0.1 MPa) may correspond to a hyperphysiologic magnitude of tissue strain.

The release of COMP into the media increased with increasing magnitude of mechanical stress at magnitudes greater than 0.025 MPa. This increased COMP may have resulted from facilitated release due to dynamic loading or degradation of COMP, rather than increased biosynthesis as the overall matrix biosynthetic rates did not increase with increasing stress. Our data, in conjunction with previous studies showing increased COMP gene expression following cyclic compression of bovine articular cartilage explants[61,63], support the hypothesis that the synthesis and release of COMP in cartilage can be altered by mechanical compression. Furthermore, a recent study has shown increased serum COMP levels in healthy subjects following 30 min of walking[59], providing *in vivo* supporting evidence that local mechanical factors influence COMP production and release from the articular cartilage.

Total S-GAG release into the media changed in a pattern that depended on the magnitude of stress, with significant increases overall following mechanical loading except at the lowest stress (0.006 MPa). The release of S-GAG from cartilage explants reflects a combination of new matrix synthesis, proteoglycan degradation, and macromolecular transport from the tissue into the media. Our data are in agreement with previous studies showing significant increases of S-GAG release into the media following low magnitudes of cyclic[47], intermittent[45,72], and traumatic[50] mechanical loading, and into synovial fluids in canine[28] and human[27] OA.

In this study, KS release into the media, represented by the 5D4 epitope, changed in a bimodal pattern that was similar to that of the total S-GAG response to the magnitude of applied stress. At a stress magnitude of 0.05 MPa, associated with inhibition of protein and proteoglycan synthesis, KS was increased significantly, suggesting that

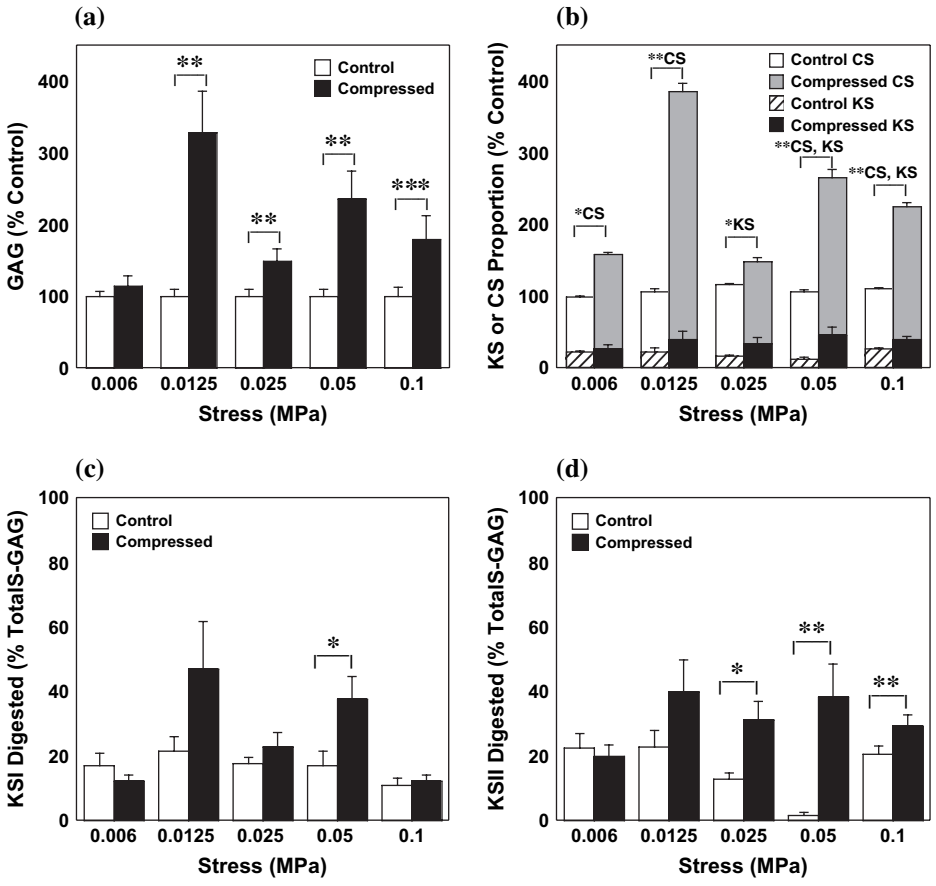

Fig. 4. The effects of mechanical compression at 0.006–0.1 MPa on (a) S-GAG release; (b) proportion of total S-GAG release in the culture media due to CS and KS via enzymatic digestion with 37.5 mU chondroitinase ABC or 37.5 mU of KI and 6.5 mU of KII, respectively; (c) proportion of total S-GAG release following enzymatic digestion with 37.5 mU of KI alone; and (d) proportion of total S-GAG release following enzymatic digestion with 6.5 mU of KII alone. Results are normalized to wet weight of the articular cartilage explants and expressed as the percent change of compressed over control values. Data are presented as mean ± S.E.M., $n = 18$, $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.01$.

facilitated release due to dynamic loading or aggrecan catabolism, rather than increased proteoglycan synthesis was responsible for the observed effect. In addition, our findings showed that the fraction of KS digestible by KI behaved differently from that digestible by KII with increasing mechanical loading, indicating differences in the amounts of mono- and disulfated disaccharides present. Previous studies have characterized the disaccharide

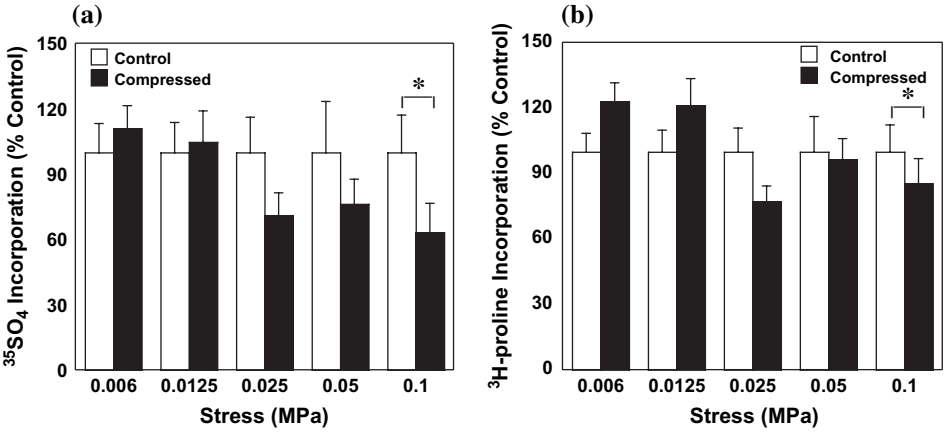

Fig. 5. (a) Proteoglycan synthesis and (b) protein synthesis measured by the uptake of $^{35}SO_4$ and ^{3}H-proline, respectively, by cartilage explants following mechanical loading of 0.006–0.1 MPa at 0.5 Hz. Results are normalized to wet weight of the articular cartilage explants and expressed as the percent change of compressed over control values. Data are presented as mean ± S.E.M., $n = 18$, $^{*}P < 0.05$.

composition[73] and level of sulfation of KS and determined that the ratio of unsulfated:monosulfated:disulfated disaccharides in the KS-rich regions of aggrecan is approximately 1:3:5[74]. The digestible fractions tested in this study may represent different pools of aggrecan KS, whose release is differentially regulated by different levels of mechanical load. Due to the differences in composition and mechanical properties of the extracellular matrix, compressive loading will likely result in a non-uniform stress–strain state in the tissue[75–77], and the observed differences in biomarker release may reflect the influence of mechanical stress on chondrocytes from different zones within the articular cartilage[77].

In summary, we observed decreased biosynthesis rates and increased matrix degradation at the highest stress applied (0.1 MPa). These findings are consistent with previous observations that cyclic tensile strains of low magnitude (3–8% equibiaxial strain) and physiological levels of cyclic compressive forces (15% compression) elicit an anabolic response[78–83], while strains of high magnitude (10–15% equibiaxial strain) initiate cartilage damage[78,79]. While the mechanisms involved in these processes are not fully understood, our findings indicate that mechanical factors can increase the production and/or release of several OA-related biomarkers. In this study, a dose–response relationship between mechanical stress and OA-related biomarker release was strongest for COMP. Moreover, the differences in the patterns of biomarker release with stress magnitude, namely between COMP and total S-GAG, and the KI- and KII-digestible fractions of KS, suggest that the magnitude of stress applied to the articular cartilage may differentially regulate chondrocytes from different zones and thus the turnover of distinct pools of molecules in the cartilage extracellular matrix.

Acknowledgments

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References

1. Howell DS, Treadwell BV, Trippel SB. Etiopathogenesis of osteoarthritis. In: Moskowitz RW, Howell DS, Goldberg VM, Mankin HJ, Eds. Osteoarthritis, Diagnosis and Medical/Surgical Management. Philadelphia: W.B. Saunders 1992;233–52.
2. Mankin HJ, Brandt KD. Biochemistry and metabolism of articular cartilage in osteoarthritis. In: Moskowitz RW, Howell DS, Goldberg VM, Mankin HJ, Eds. Osteoarthritis: Diagnosis and Medical/Surgical Management. Philadelphia: W.B. Saunders 1992;109–54.
3. Poole AR, Rizkalla G, Ionescu M, Reiner A, Brooks E, Rorabeck C, *et al*. Osteoarthritis in the human knee: a dynamic process of cartilage matrix degradation, synthesis and reorganization. Agents Actions Suppl 1993;39:3–13.
4. Poole AR, Nelson F, Hollander A, Reiner A, Pidoux I, Ionescu M. Collagen II turnover in joint diseases. Acta Orthop Scand Suppl 1995;266:88–91.
5. Eyre DR. The collagens of articular cartilage. Semin Arthritis Rheum 1991;21:2–11.
6. Hardingham TE, Fosang AJ, Dudhia J. The structure, function and turnover of aggrecan, the large aggregating proteoglycan from cartilage. Eur J Clin Chem Clin Biochem 1994;32:249–57.
7. Ratcliffe A, Flatow EL, Roth N, Saed-Nejad F, Bigliani LU. Biochemical markers in synovial fluid identify early osteoarthritis of the glenohumeral joint. Clin Orthop 1996:45–53.
8. Heinegård D, Oldberg A. Structure and biology of cartilage and bone matrix noncollagenous macromolecules. FASEB J 1989;3:2042–51.
9. Hazell PK, Dent C, Fairclough JA, Bayliss MT, Hardingham TE. Changes in glycosaminoglycan epitope levels in knee joint fluid following injury. Arthritis Rheum 1995;38:953–9.
10. Lindhorst E, Vail TP, Guilak F, Wang H, Setton LA, Vilim V, *et al*. Longitudinal characterization of synovial fluid biomarkers in the canine meniscectomy model of osteoarthritis. J Orthop Res 2000;18:269–80.
11. Saxne T, Glennas A, Kvien TK, Melby K, Heinegard D. Release of cartilage macromolecules into the synovial fluid in patients with acute and prolonged phases of reactive arthritis. Arthritis Rheum 1993;36:20–5.
12. Saxne T. Differential release of molecular markers in joint disease. Acta Orthop Scand Suppl 1995;266: 80–3.
13. Caterson B, Mahmoodian F, Sorrell JM, Hardingham TE, Bayliss MT, Carney SL, *et al*. Modulation of native chondroitin sulphate structure in tissue development and in disease. J Cell Sci 1990;97:411–7.
14. Oldberg A, Antonsson P, Lindblom K, Heinegard D. COMP (cartilage oligomeric matrix protein) is structurally related to the thrombospondins. J Biol Chem 1992;267:22346–50.
15. Lohmander LS, Saxne T, Heinegard DK. Release of cartilage oligomeric matrix protein (COMP) into joint fluid after knee injury and in osteoarthritis. Ann Rheum Dis 1994;53:8–13.
16. Sharif M, Saxne T, Shepstone L, Kirwan JR, Elson CJ, Heinegard D, *et al*. Relationship between serum cartilage oligomeric matrix protein levels and disease progression in osteoarthritis of the knee joint. Br J Rheumatol 1995;34:306–10.
17. Jordan JM, Luta G, Stabler T, Renner JB, Dragomir AD, Vilim V, *et al*. Ethnic and sex differences in serum levels of cartilage oligomeric matrix protein: the Johnston County Osteoarthritis Project. Arthritis Rheum 2003;48:675–81.
18. Clark AG, Jordan JM, Vilim V, Renner JB, Dragomir AD, Luta G, *et al*. Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity: the Johnston County Osteoarthritis Project. Arthritis Rheum 1999;42:2356–64.
19. Vilim V, Vytasek R, Olejarova M, Machacek S, Gatterova J, Prochazka B, *et al*. Serum cartilage oligomeric matrix protein reflects the presence of clinically diagnosed synovitis in patients with knee osteoarthritis. Osteoarthritis Cartilage 2001;9:612–8.
20. Vilim V, Olejarova M, Machacek S, Gatterova J, Kraus VB, Pavelka K. Serum levels of cartilage oligomeric

matrix protein (COMP) correlate with radiographic progression of knee osteoarthritis. Osteoarthritis Cartilage 2002;10:707–13.
21. Vilim V, Voburka Z, Vytasek R, Senolt L, Tchetverikov I, Kraus VB, *et al*. Monoclonal antibodies to human cartilage oligomeric matrix protein: epitope mapping and characterization of sandwich ELISA. Clin Chim Acta 2003;328:59–69.
22. Garnero P, Rousseau JC, Delmas PD. Molecular basis and clinical use of biochemical markers of bone, cartilage, and synovium in joint diseases. Arthritis Rheum 2000;43:953–68.
23. Thonar EJ, Lenz ME, Klintworth GK, Caterson B, Pachman LM, Glickman P, *et al*. Quantification of keratan sulfate in blood as a marker of cartilage catabolism. Arthritis Rheum 1985;28:1367–76.
24. Williams JM, Downey C, Thonar EJ. Increase in levels of serum keratan sulfate following cartilage proteoglycan degradation in the rabbit knee joint. Arthritis Rheum 1988;31:557–60.
25. Manicourt DH, Lenz ME, Thonar EJ. Levels of serum keratan sulfate rise rapidly and remain elevated following anterior cruciate ligament transection in the dog. J Rheumatol 1991;18:1872–6.
26. Spector TD, Woodward L, Hall GM, Hammond A, Williams A, Butler MG, *et al*. Keratan sulphate in rheumatoid arthritis, osteoarthritis, and inflammatory diseases. Ann Rheum Dis 1992;51:1134–7.
27. Lohmander LS, Dahlberg L, Ryd L, Heinegard D. Increased levels of proteoglycan fragments in knee joint fluid after injury. Arthritis Rheum 1989;32: 1434–42.
28. Ratcliffe A, Beauvais PJ, Saed-Nejad F, Shurety W, Caterson B. Synovial fluid analyses detect and differentiate proteoglycan metabolism in canine experimental models of osteoarthritis and disuse atrophy. Agents Actions Suppl 1993;39:63–7.
29. Buckwalter JA, Martin J. Degenerative joint disease. Clin Symp 1995;47:1–32.
30. Sharma L. Examination of exercise effects on knee osteoarthritis outcomes: why should the local mechanical environment be considered? Arthritis Rheum 2003;49:255–60.
31. Sharma L. The role of proprioceptive deficits, ligamentous laxity, and malalignment in development and progression of knee osteoarthritis. J Rheumatol Suppl 2004;70:87–92.
32. Felson DT, Chaisson CE. Understanding the relationship between body weight and osteoarthritis. Baillieres Clin Rheumatol 1997;11:671–81.
33. Helminen HJ, Jurvelin J, Kiviranta I, Paukkonen K, Säämänen AM, Tammi M. Joint loading effects on articular cartilage: a historical review. In: Helminen HJ, Kiviranta I, Tammi M, Säämänen AM, Paukkonen K, Jurvelin J, Eds. Joint Loading: Biology and Health of Articular Structures. Bristol: Wright and Sons 1987;1–46.
34. Messier SP. Osteoarthritis of the knee and associated factors of age and obesity: effects on gait. Med Sci Sports Exerc 1994;26:1446–52.
35. Guilak F, Meyer BC, Ratcliffe A, Mow VC. The effects of matrix compression on proteoglycan metabolism in articular cartilage explants. Osteoarthritis Cartilage 1994;2:91–101.
36. O'Connor BL, Visco DM, Heck DA, Myers SL, Brandt KD. Gait alterations in dogs after transection of the anterior cruciate ligament. Arthritis Rheum 1989;32: 1142–7.
37. Setton LA, Mow VC, Muller FJ, Pita JC, Howell DS. Mechanical properties of canine articular cartilage are significantly altered following transection of the anterior cruciate ligament. J Orthop Res 1994;12:451–63.
38. Gray ML, Pizzanelli AM, Grodzinsky AJ, Lee RC. Mechanical and physiochemical determinants of the chondrocyte biosynthetic response. J Orthop Res 1988;6:777–92.
39. Larsson T, Aspden RM, Heinegard D. Effects of mechanical load on cartilage matrix biosynthesis *in vitro*. Matrix 1991;11:388–94.
40. Lin PM, Chen CT, Torzilli PA, Grigiene R. Increased stromelysin-1 (MMP-3), proteoglycan degradation (3B3- and 7D4) and collagen damage in cyclically load-injured articular cartilage. Osteoarthritis Cartilage 2004;12:485–96.
41. Ostendorf RH, van de Stadt RJ, van Kampen GP. Intermittent loading induces the expression of 3-B-3(-) epitope in cultured bovine articular cartilage. J Rheumatol 1994;21:287–92.
42. Palmoski MJ, Brandt KD. Effects of static and cyclic compressive loading on articular cartilage plugs *in vitro*. Arthritis Rheum 1984;27:675–81.
43. Steinmeyer J, Knue S. The proteoglycan metabolism of mature bovine articular cartilage explants superimposed to continuously applied cyclic mechanical loading. Biochem Biophys Res Commun 1997;240:216–21.
44. Sah RL, Kim YJ, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD. Biosynthetic response of cartilage explants to dynamic compression. J Orthop Res 1989;7: 619–36.
45. Sah RL, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD. Effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage. Arch Biochem 1991;286:20–9.
46. Torzilli PA, Grigiene R. Continuous cyclic load reduces proteoglycan release from articular cartilage. Osteoarthritis Cartilage 1998;6:260–8.
47. Thibault M, Poole AR, Buschmann MD. Cyclic compression of cartilage/bone explants *in vitro* leads to physical weakening, mechanical breakdown of collagen and release of matrix fragments. J Orthop Res 2002;20:1265–73.
48. Chen CT, Bhargava M, Lin PM, Torzilli PA. Time, stress, and location dependent chondrocyte death and collagen damage in cyclically loaded articular cartilage. J Orthop Res 2003;21:888–98.
49. DiMicco MA, Patwari P, Siparsky PN, Kumar S, Pratta MA, Lark MW, *et al*. Mechanisms and kinetics of glycosaminoglycan release following *in vitro* cartilage injury. Arthritis Rheum 2004;50:840–8.
50. D'Lima DD, Hashimoto S, Chen PC, Colwell CW Jr, Lotz MK. Impact of mechanical trauma on matrix and cells. Clin Orthop 2001:S90–9.
51. Morel V, Quinn TM. Short-term changes in cell and matrix damage following mechanical injury of articular cartilage explants and modelling of microphysical mediators. Biorheology 2004;41:509–19.
52. Patwari P, Gaschen V, James IE, Berger E, Blake SM, Lark MW, *et al*. Ultrastructural quantification of cell death after injurious compression of bovine calf articular cartilage. Osteoarthritis Cartilage 2004;12: 245–52.
53. Guilak F, Sah RL, Setton LA. Physical regulation of cartilage metabolism. In: Mow VC, Hayes WC, Eds. Basic Orthopaedic Biomechanics. Philadelphia: Lippincott-Raven 1997;179–207.

54. Roos H, Dahlberg L, Hoerrner LA, Lark MW, Thonar EJ, Shinmei M, *et al*. Markers of cartilage matrix metabolism in human joint fluid and serum: the effect of exercise. Osteoarthritis Cartilage 1995;3:7–14.
55. Okumura M, Kim GH, Tagami M, Haramaki S, Fujinaga T. Serum keratan sulphate as a cartilage metabolic marker in horses: the effect of exercise. J Vet Med A Physiol Pathol Clin Med 2002;49:195–7.
56. Sweet MB, Jakim I, Coelho A, Becker PJ, Thonar EJ. Serum keratan sulfate levels in marathon runners. Int J Sports Med 1992;13:348–50.
57. Neidhart M, Muller-Ladner U, Frey W, Bosserhoff AK, Colombani PC, Frey-Rindova P, *et al*. Increased serum levels of non-collagenous matrix proteins (cartilage oligomeric matrix protein and melanoma inhibitory activity) in marathon runners. Osteoarthritis Cartilage 2000;8:222–9.
58. Kraus VB, Gell N, Blumenthal J. The effect of chronic exercise on circulating biomarkers in individuals with musculoskeletal disease. Clin Ex Phys 1999;1:17–23.
59. Munderman A, Dyrby CO, Andriacchi TP, King KB. Serum concentration of cartilage oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults. Osteoarthritis Cartilage 2005;13:34–8.
60. Chu Q, Lopez M, Hayashi K, Ionescu M, Billinghurst RC, Johnson KA, *et al*. Elevation of a collagenase generated type II collagen neoepitope and proteoglycan epitopes in synovial fluid following induction of joint instability in the dog. Osteoarthritis Cartilage 2002;10:662–9.
61. Wong M, Siegrist M, Cao X. Cyclic compression of articular cartilage explants is associated with progressive consolidation and altered expression pattern of extracellular matrix proteins. Matrix Biol 1999;18: 391–9.
62. Wong M, Siegrist M, Goodwin K. Cyclic tensile strain and cyclic hydrostatic pressure differentially regulate expression of hypertrophic markers in primary chondrocytes. Bone 2003;33:685–93.
63. Giannoni P, Siegrist M, Hunziker EB, Wong M. The mechanosensitivity of cartilage oligomeric matrix protein (COMP). Biorheology 2003;40:101–9.
64. Fermor B, Weinberg JB, Pisetsky DS, Misukonis MA, Banes AJ, Guilak F. The effects of static and intermittent compression on nitric oxide production in articular cartilage explants. J Orthop Res 2001;19: 729–37.
65. Fermor B, Weinberg JB, Pisetsky DS, Misukonis MA, Fink C, Guilak F. Induction of cyclooxygenase-2 by mechanical stress through a nitric oxide-regulated pathway. Osteoarthritis Cartilage 2002;10:792–8.
66. Fink C, Fermor B, Weinberg JB, Pisetsky DS, Misukonis MA, Guilak F. The effect of dynamic mechanical compression on nitric oxide production in the meniscus. Osteoarthritis Cartilage 2001;9:481–7.
67. Kraus VB, Huebner JL, Stabler T, Flahiff CM, Setton LA, Fink C, *et al*. Ascorbic acid increases the severity of spontaneous knee osteoarthritis in a guinea pig model. Arthritis Rheum 2004;50:1822–31.
68. Farndale RW, Sayers CA, Barrett AJ. A direct spectrophotometric microassay for sulfated glycosaminoglycans in cartilage cultures. Connect Tissue Res 1982;9: 247–8.
69. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochem Biophys Acta 1986;883:173–7.
70. Armstrong CG, Bahrani AS, Gardner DL. *In vitro* measurement of articular cartilage deformations in the intact human hip joint under load. J Bone Joint Surg 1979;61:744–55.
71. Eckstein F, Lemberger B, Stammberger T, Englmeier KH, Reiser M. Patellar cartilage deformation *in vivo* after static versus dynamic loading. J Biomech 2000; 33:819–25.
72. Sauerland K, Raiss RX, Steinmeyer J. Proteoglycan metabolism and viability of articular cartilage explants as modulated by the frequency of intermittent loading. Osteoarthritis Cartilage 2003;11:343–50.
73. Plaas AH, West LA, Midura RJ. Keratan sulfate disaccharide composition determined by FACE analysis of keratanase II and endo-beta-galactosidase digestion products. Glycobiology 2001;11:779–90.
74. Fosang AJ, Last K, Plaas AH, Poon CJ. Keratan sulphate in the aggrecan interglobular domain has a microstructure that is distinct from keratan sulphate elsewhere on aggrecan. Trans Orthop Res Soc 2004; 29:232.
75. Guilak F, Mow VC. The mechanical environment of the chondrocyte: a biphasic finite element model of cell–matrix interactions in articular cartilage. J Biomech 2000;33:1663–73.
76. Schinagl RM, Gurskis D, Chen AC, Sah RL. Depth-dependent confined compression modulus of full-thickness bovine articular cartilage. J Orthop Res 1997;15:499–506.
77. Wong M, Carter DR. Articular cartilage functional histomorphology and mechanobiology: a research perspective. Bone 2003;33:1–13.
78. Gassner R, Buckley MJ, Georgescu H, Studer R, Stefanovich-Racic M, Piesco NP, *et al*. Cyclic tensile stress exerts antiinflammatory actions on chondrocytes by inhibiting inducible nitric oxide synthase. J Immunol 1999;163:2187–92.
79. Xu Z, Buckley MJ, Evans CH, Agarwal S. Cyclic tensile strain acts as an antagonist of IL-1 beta actions in chondrocytes. J Immunol 2000;165:453–60.
80. Long P, Gassner R, Agarwal S. Tumor necrosis factor alpha-dependent proinflammatory gene induction is inhibited by cyclic tensile strain in articular chondrocytes *in vitro*. Arthritis Rheum 2001;44:2311–9.
81. Agarwal S, Long P, Gassner R, Piesco NP, Buckley MJ. Cyclic tensile strain suppresses catabolic effects of interleukin-1beta in fibrochondrocytes from the temporomandibular joint. Arthritis Rheum 2001;44:608–17.
82. Lee DA, Frean SP, Lees P, Bader DL. Dynamic mechanical compression influences nitric oxide production by articular chondrocytes seeded in agarose. Biochem Biophys Res Commun 1998;251:580–5.
83. Lee DA, Noguchi T, Frean SP, Lees P, Bader DL. The influence of mechanical loading on isolated chondrocytes seeded in agarose constructs. Biorheology 2000;37:149–61.